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INFLUENCES OF THE LATERAL GENICULATE NUCLEUS  
IN THE SPECIFICATION OF PRIMARY VISUAL CORTEX  
IN MACACA MULATTA

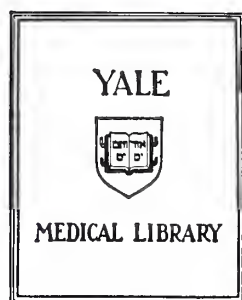
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Ivan Jose Suner

Yale University

1992







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**INFLUENCES OF THE LATERAL GENICULATE NUCLEUS**  
**IN THE SPECIFICATION OF PRIMARY VISUAL CORTEX**  
**IN *MACACA MULATTA***

A thesis submitted to the Yale University School of Medicine  
in partial fulfillment of the requirements  
for the degree of  
Doctor in Medicine

by  
Iván José Suárez  
May 1992

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*No natural phenomenon can be adequately studied in itself alone,  
but to be understood must be considered as it stands connected with all nature.*

-Sir Francis Bacon





## ABSTRACT

The cerebral cortex is composed of morphologically and functionally distinct cytoarchitectonic areas. The influences of thalamic afferents in the specification of cytoarchitectonic patterns and area size in the primate cerebral cortex were explored by studying relationships between lateral geniculate nucleus (LGN) and primary visual cortex (area 17) in macaque monkeys in (1) normal conditions and (2) after experimental reduction of LGN input during perinatal development.

The relationship between total populations of neurons in LGN and area 17 was analyzed in five normal adults. Results showed consistent right-left asymmetry in number of neurons of LGN and area 17 that favored the right side in 80% of subjects. However, because of small variation across subjects in total neuron number in area 17 ( $341 \pm 54$  million) and LGN ( $1.4 \pm 0.2$  million), a strong correlation was not observed ( $r^2=0.29$ ). Lack of variability in either size or number of neurons in these visual structures suggests their coordination in development and a possible role for extrinsic factors such as thalamic afferents in specification of cerebral cortex.

In the second study, cytoarchitectonic patterns and boundaries for area 17 were examined in five macaques with experimentally-reduced number of neurons in LGN due to binocular enucleation at various stages of embryonic development; following *in utero* neurosurgery, embryos were returned to womb and carried to term --embryonic day 165 (E165)-- and sacrificed. The surface area and neuron number of area 17 in animals operated between E59 and E90 were significantly reduced compared to controls. Those operated on E81 and E90 displayed a novel, "hybrid" area distinguishable from areas 17 and 18 --termed area X-- within area 17 and at its border. These results support the protomap hypothesis in which basic, species-specific cytoarchitectonic features of cortical areas are intrinsically determined, but may be modified by extrinsic factors. This model is helpful for understanding possible mechanisms of various genetic and acquired pathological alterations of cortical organization and provides insights into the evolution of parcellation in cerebral cortex.



## **CHAPTER 1: General Introduction**





The human cerebral cortex is one of the most remarkable products of Nature. It allows us to perceive our surroundings by means of the various sensory modalities: vision, hearing, touch, taste, and smell. In turn, it enables us to process these perceptions for analysis, integration, and coordination of responses. The brain also endows us with the uniquely human capacities of abstract thought, emotion, personality, and language.

Many of these functions may be traced to specific cortical areas and their specific higher-order association areas. Thus, the cortex is parcelled into various functional areas with sensory, motor, cognitive, and integrative functions. Recently, there has been great interest in how parcellation arises in the neocortex (Rakic, 1988, 1990a; O'Leary, 1989; Killackey, 1990; Jones, 1990; Windrem and Finlay, 1991); specifically, to what extent is cortical specification dictated by intrinsic properties, and to what extent are extrinsic influences --such as size and pattern of afferent and efferent connections, tropic systemic factors-- involved in cortical parcellation. Is cerebral cortex homogeneous across its entire surface during development or is it innately regionally-specified? To which extent are area differences genetically "hard-wired" and what is the role for epigenetic and environmental influences? Are these determinants of cortical parcellation consistent with evolutionary models for differences in function, degree of development, and relative sizes of these areas across species?

In this work, we explore the influence of specific thalamo-cortical interactions on laminar and areal differentiation of primate primary visual cortex. Through these studies we attempt to add to our understanding of the role of epigenetic interactions in the normal development of the cerebral cortex, as well as in certain pathologic conditions.

Chapter 1 provides a general introduction to development in the cerebral cortex and describes specific timing of developmental events in the primate visual system. Also, it discusses current theories for the specification of cortical areas.



Chapter 2 examines the correlation between total neuron number in lateral geniculate nucleus (LGN) and the corresponding hemisphere of primary visual cortex (area 17) to which it projects in normal rhesus monkeys. These relationships are described for the two hemispheres of the same animal and for the population as a whole.

Chapter 3 examines the effects of prenatal reduction of geniculocortical neuron number on the development and specification of area 17 lamination and surface area.

### **Differences and Similarities in Neocortical Areas**

The primate cerebral cortex has been divided into morphologically-distinct regions (Brodman, 1909; Krieg, 1946). These areas have been further characterized by particular cortical lamination patterns, cytoarchitectonic patterns, distributions of cell types and neurotransmitter receptors, afferent and efferent connections, and physiological properties (Peters and Jones, 1984; Rakic and Singer, 1988). Thus, it is likely that these area-specific characteristics endow cortical areas with their unique functional characteristics.

Despite these regional differences, there are unifying themes in the functional organization of the cerebral cortex. All neocortical areas are composed of six primary layers. Furthermore, there is remarkable fidelity in the organization and function of these laminae across cortical areas. The superficial layers, II and III, communicate mainly with ipsilateral and contralateral neocortical targets; they also contain most of the local circuit neurons. Layer IV, the middle layer, receives input from the thalamic nuclei and makes connections primarily with the other laminae. Meanwhile, the deeper layers, V and VI, project to subcortical structures; layer V sends fibers to various subcortical targets, while layer VI sends input to the thalamus and claustrum (Gilbert, 1983; Peters and Jones, 1984).

The number of neurons in a radial transverse unit of cortex is remarkably constant from area to area within species as well as across species (Rockel *et al.*, 1980; Finlay and Slattery, 1983). The only clear exception is in primate primary visual cortex (area 17), in



which the number of neurons per radial transverse is consistently higher than in other areas (Rockel *et al.*, 1980; O'Kusky and Colonnier, 1982). Although these initial findings may be overgeneralized (Williams and Rakic, 1989), they nevertheless provide a basic framework for our understanding of cytoarchitectonic organization.

Thus, neocortical areas display a common organization and pattern of connections. Permutations within these general anatomical and structural schemes may result in the differences that are characteristic of the various cortical functional areas.

### **Neurogenesis Occurs Prenatally in Primates**

Cortical neurons in human and nonhuman primates are generated before birth. This was demonstrated in monkeys by Rakic in elegant experiments using  $^3\text{H}$ -thymidine labelling to mark DNA replication stages (Rakic, 1974; Rakic, 1977). These studies revealed precise parameters for the initiation and termination of cortical neurogenesis. In general, neurogenesis occurs during midgestation, beginning approximately at embryonic day 40 (E40) with gestation spanning 165 days in monkeys; it ends earliest in limbic cortex (area 24) at E70, and latest in primary visual cortex (area 17) at E100 (Rakic, 1977).

Similarly, in humans area 17 cortical neurons are generated from E40 to E125, with the gestational period spanning 265 days (Rakic, 1978). This is in sharp contrast to most other mammalian species in which corticogenesis extends into the postnatal period (Angevine and Sidman, 1961; Berry and Rogers, 1965; Caviness, 1982; Smart and Smart, 1982; Luskin and Shatz, 1985a). Therefore it seems clear that in primates initial subdivision of the cortex into cytoarchitectonic areas occurs prenatally, well before significant sensory stimulation occurs.

### **The Development of Cerebral Cortex**

Cortical neurons are generated in the neuroepithelium of the ventricular zone. This was first suggested by Ramón y Cajal upon the observation of numerous mitotic figures at





the ventricular surface of human fetal cerebrum (Ramón y Cajal, 1911), and later confirmed by  $^3\text{H}$ -thymidine labelling studies (Sidman, Miale, and Feder, 1959; Rakic, 1975).

These precursor neurons arise in rhesus monkeys in the pseudostratified neuroepithelium of the ventricular zone at approximately E40. They divide asynchronously with the nuclei mobilizing away from the ventricular surface during the DNA synthetic stage, and subsequently returning to the ventricular surface and undergoing a second mitotic division (Sidman, Miale, and Feder, 1959; Rakic, 1975). These precursor cells demarcate columns within the ventricular zone that are termed "proliferative units" (Rakic, 1978).

Each proliferative unit consists of several clones of dividing cells and gives rise to cohorts of postmitotic neurons that migrate away from the ventricular zone along the shafts of radial glial cells (Rakic, 1971; Rakic, 1972). It is thought that gliophilic or recognition molecules at the surface of elongated radial fibers result in the high fidelity of neuronal migration along these physical guides (Rakic, 1981, 1990b).

Radial glial guides extend through the transient intermediate and subplate zones on their course to the expanding fetal cerebral wall. These transient zones constitute the earliest cellular structures in the developing cortex (Sidman and Rakic, 1973). As neurons migrate through these zones, they come into contact with early afferent fibers from the thalamus (Rakic, 1976) and other cortical areas (Wise and Jones, 1976; Goldman-Rakic, 1982). Transient synaptic and neurotransmitter/neuromodulator patterns appear during the generation and migration of neurons before these zones disappear (Kostovic and Rakic, 1980; Rakic, 1982; Luskin and Shatz, 1985b). Subsequent studies have suggested that subplate may be necessary for development of the cortical plate (Chun, Nakamura, and Shatz, 1987). Thus, the subplate zone may serve the role of a waiting compartment for these afferent fibers, allowing for interactions with migrating neurons before they reach the cortical plate (Rakic, 1977; Shatz, Chun, and Luskin, 1988; Kostovic and Rakic, 1990).



Migrating neurons populate the cortical plate in an inside-out fashion; that is, younger neurons occupy progressively more superficial positions in the cortical plate in a gradient progressing from deep to superficial in layer VI to layer I. This has been shown in a variety of mammalian species (Angevine and Sidman, 1961; Berry and Rogers, 1969; Luskin and Shatz, 1985a), but is particularly prominent in primates (Rakic, 1974).

### **Theories for the Specification of Cortical Areas**

There are various theories that attempt to account for the development of regional specialization within the cerebral cortex. These theories span a continuum from that of total intrinsic determination of cortical parcellation to that of total specification by extrinsic induction of parcellation by epigenetic mechanisms.

Total intrinsic genetic determination implies that the neuroepithelium of the ventricular zone produces area-unique lineages of neurons endowed with unalterable genetic programs for area specificity, neuronal subtypes, and synaptic organization. This theory currently has few if any supporters, as experimental evidence has clearly shown that interaction with afferents must play an important role, which is substantiated by studies reviewed in the next section.

The theory of a homogeneous ventricular neuroepithelium that subsequently becomes specified by epigenetic mechanisms is one of the two current leading theories of cortical parcellation. Proponents of this view argue that there is phylogenetic conservation in developing neocortex; thus, the neuroepithelium of the ventricular zone is totally pluripotent, devoid of any regional specificity until extrinsic influences, such as contact with afferent connections, promote differentiation of neurons during migration and/or at their final destination in the cortical plate. The implication is that neurons in the ventricular zone of the embryo destined for a particular cortical area have the potential to acquire area-specific characteristics of any other areas depending on epigenetic mechanisms such as contact with specific thalamic afferents (O'Leary, 1989).





The current competing theory --the protomap hypothesis (Rakic, 1988)-- attributes roles to both genetic determination and epigenetic factors in regulating cortical parcellation. The basic premise is that the neuroepithelium of the ventricular zone defines a basic species-specific protomap for cortical areas; that is, it lays area-specific foundations for parcellation that interact with and may be altered by epigenetic mechanisms. Thus, this theory implies a synergism between these two influences. It is grounded on the radial unit hypothesis, which proposes vertical units defined by progenitor neurons at the ventricular zone as the building blocks of neocortex (Rakic, 1978, Rakic, 1988c). This theory will be examined in the next section, while evidence challenging the radial unit model will follow.

### **The Radial Unit Hypothesis**

The radial unit hypothesis proposes a model for development in which columnar units serve as the building blocks of cortex (Rakic, 1988b). Initially, neuronal progenitor cells in the ventricular zone divide symmetrically to form other progenitor cells (Rakic, 1975). However, after approximately E40 each progenitor cell proceeds to divide asymmetrically; one daughter cell becomes a migrating neuron, while the other remains a stem cell or becomes inviable (Rakic, 1988c). These migrating neurons eventually reach the cortical plate and become arranged in stacks perpendicular to the cortical surface; these stacks are termed ontogenetic columns, as each originates arises from a single proliferative unit (Rakic, 1978). Thus, a proliferative unit defines an ontogenetic column.

Each proliferative unit must, therefore, produce a variety of neuronal phenotypes to account for the multiple cell types across ontogenetic columns. Thus, cortical neurons must commit to neuronal phenotypes post-mitotically, but before reaching their final position in the cortex. Review of the literature by Rakic in 1988 revealed compelling evidence supporting this hypothesis: 1) in monkey embryos, ventricular zone corresponding to cortex where neuron density is higher produces more neurons over a longer period than ventricular zone corresponding to cortex with lower neuron density



(Rakic, 1976b); 2) in the reeler mouse mutations, which are characterized by displacement of cortical laminae to abnormal positions, neuronal cell phenotypes correlate with temporal and positional origins rather than with final position in cortex (Caviness and Rakic, 1978); 3) disruption of neuronal migration following exposure to ionizing radiation during early development results in arrest of migration of some neurons, resulting in their final position near the ventricles; however, these displaced neurons preserve the phenotype and connectivity that would be predicted by their developmental birthdate (Jensen and Killackey, 1984); 4) neuroepithelium from ventricular zone in ferret embryo donors transplanted into telencephalon of newborn recipients develops final cortical position, phenotypes, and synaptic connectivity appropriate for the donor site (McConnell, 1985, 1988); 5) neurons destined to form corpus callosum in fetal monkeys first establish axonal contact with contralateral hemisphere before arriving in ipsilateral cortical plate (Schwartz, Rakic, and Goldman-Rakic, 1991); from this study, it is also likely that the synptoarchitecture of cortical neurons may be specified before final neuron position is determined; and 6) studies using retrovirus-labelled retinal neurons have shown that progenitors usually give rise to radially-aligned columns of neurons (Luskin, Pearlman, and Sanes, 1988; Parnavelas *et al.*, 1991; Nakatsuji *et al.*, 1991). Also, in a recent study, sensorimotor cortical neurons transplanted to homo- and heterotopic locations demonstrated that commitment to particular neuronal phenotypes --as assayed by the presence or absence of limbic-system-associated membrane protein (LAMP)-- occurs following an early pliant period (Barbe and Levitt, 1991). Another group in Japan has found a similar marker that appears early in development and disappears during migration (Arimatsu *et al.*, in press).

The number of neurons per ontogenetic column in each Brodmann area correlates well with the duration of neurogenesis. The cingulate cortex (area 24) has approximately 80 neurons per column, whereas in area 17 there are approximately 120 neurons per column; neurogenesis spans one month in the former, in contrast to two months in the latter (Rakic, 1974; Rakic, 1982). This argues strongly for the role of the number of mitotic



divisions in determining the size of final neuron populations. Differential cell death also seems to play a role in the regulation of final neuron numbers (Finlay and Slattery, 1983; Williams and Herrup, 1988).

In summary, according to the radial unit hypothesis, the foundations for cortical areas are laid out by the characteristics of area-specific proliferative units. Each unit generates a cohort of neurons that migrates to the cortical plate in an inside-out pattern via radial glial fiber guides, thus forming ontogenetic columns. These neurons achieve radial positions within the cortical laminae dependent on their time of origin, and acquire particular phenotypes and synaptic patterns prior to reaching the cortical plate. The neuroepithelium at the ventricular surface during embryonic development, therefore, represents a two-dimensional blueprint for the development of the cortical areas; it represents a protomap for the developing cortical plate. The size of a cortical area, thus, would be dependent on the number of ontogenetic columns allocated to it in the neuroepithelium.

### **Testing the Radial Unit Hypothesis**

Provocative studies to test the radial unit hypothesis have involved introduction of a retroviral marker into precursor cells during development in rats in order to observe the pattern of migration of individual cohorts and evaluate if clonal populations account for the columnar pattern of the cortex (Luskin *et al.*, 1988; Walsh and Cepko, 1988). Initial results from these studies demonstrated a considerable degree of clonality to the behavior of these neurons. Thus, most neurons that originate from the same precursors arrive in the cortical plate by radial deployment and remain established in these locations (Luskin *et al.*, 1988; Misson *et al.*, 1991). The results also confirm that they organize into coherent radial columns of neurons of common genetic origin (Nakatatsuji, 1991)

The more recent results have been difficult to interpret as one group reported widespread dispersion of retrovirus-infected clones across functional regions of the cortex



(Walsh and Cepko, 1992). There are, however, several technical and interpretational problems that must be resolved in order to take full advantage of this methodology. It was unclear whether the widely-dispersed cells were neurons or glia. If they were neurons, they may represent a population that would not persist or would be eliminated by differential cell death. Furthermore, if these neurons persist, they may represent local circuit neurons, which do not participate in establishing functional organization of the cortex through connections with cortical or subcortical targets. One would also have to reconcile these findings with those of another group, which, using similar methods, did not regularly observe dispersion of clonal populations, and described a columnar organization (Parnavelas *et al.*, 1991). Another possible limitation to the use of this technique in the rapidly-developing rat central nervous system is its limited period of progenitor cell division. Thus, application of this technique in a model where neurogenesis comprised a more extended period, and analysis past the time of selective cell death may provide more clear-cut results. Such experiments are now in progress employing a primate model to address these particular issues (Rakic, personal communication).

### **Testing Theories of Cortical Development by Alteration of Normal Cortical Organization During Ontogeny**

The evidence presented above in support of the radial unit hypothesis implies that the fundamental basis of cytoarchitectonic field organization is set by mechanisms that are "intrinsic," or not dependent on the presence of afferent connections. It implies, rather, that afferent connections, or "extrinsic" factors, however, may participate in the determination of field size and subareal structure. However, a number of experiments have demonstrated alteration of cortical organization.

The first series of experiments involved studies in which retinal projections in the hamster were deprived of their normal target nuclei in thalamus, resulting in invasion of adjacent nuclei (Frost, 1981) and establishment of anomalous visual connections with the





normal cortical targets of the invaded nuclei (Frost, 1982). Subsequent experiments showed visual processing to occur in these anomalously-innervated cortical areas, which included the somatosensory cortex (Frost and Metin, 1985), and the auditory cortex (Sur *et al.*, 1988).

A second series of experiments involved hetero- and homochronic transplants of specific sets of cells in developing cortex. One set of experiments involved the transplantation of specific populations of cells from the ferret visual cortex destined to become part of layers V and VI --on the basis of date of genesis-- back to the visual cortex at a time when neurons destined for layers II and III were being generated (McConnell, 1985, 1988). More than 50% of these neurons migrated to layers V and VI -- the laminae predicted from their date of genesis. However, others migrated to layers II and III. It is unclear, however, whether the cells migrating to layers II and III --the layers specific for the time of transplantation-- were actually progenitors that underwent further rounds of division.

A final set of experiments involved transplantation of cortical plate neurons from rat visual cortex to sensorimotor cortex (O'Leary and Stanfield, 1989) These transplants were taken following the completion of migration but before establishment of the cytoarchitectonic field structure. It was observed that these neurons were incorporated into the recipient areas, adopting their patterns of connectivity. It is unclear, however, whether these transplants represented a population of layer V visual cortex neurons that normally sends transient projections to spinal cord.

### **The Experimental Model: The Primate Visual System**

In the experiments herein described, we investigate the role of epigenetic influences in the specification and development of cortical areas; specifically, we address these questions with relation to thalamic input. Thalamic fibers, by virtue of their dynamic positional changes during development, are thought to be one of the major extrinsic



influences in cortical specification. These fibers are present in the subplate zone during neurogenesis and migration, where they are hypothesized to interact with migrating neurons (Rakic, 1977; Shatz *et al.*, 1988; Kostovic and Rakic, 1990). They are also present when migration to the cortical plate is finalized, where they provide afferent input to the cortex and also receive input from cortical areas.

We used the rhesus monkey visual system as our experimental model. This system is ideal for a variety of reasons: 1) there is great fidelity in the primate visual system with over 90% of retinal input innervating LGN (Polyak, 1957; and Perry *et al.*, 1984), and over 90% of LGN input innervating area 17 (Norden and Kaas, 1978; Pasik *et al.*, 1986); 2) LGN serves as the major input to area 17; in turn, the LGN is a major target of cortical afferents from layer VI of area 17 (Polyak, 1957); 3) the characteristic cytoarchitecture of normal primate area 17 allows for clear demarcation of its areal boundaries (Brodmann, 1905; Polyak, 1957); 3) the layer-specific patterns of neuronal types and connections in primate area 17 is well characterized (reviewed in Valverde, 1985); 4) the timing of events in the development of primary visual cortex in primates is known with a great degree of precision (Rakic, 1974, 1977); and 5) there is a strong anatomic correlation between macaque's visual system and that of humans (Polyak, 1957).

We decided to study influences of geniculocortical connections in both normal conditions and experimentally-altered conditions. Experimental manipulation of cortical development provides the challenges to the normal system that are often needed to obtain differences --be they qualitative or quantitative-- that are sometimes not readily seen in Nature. The limitations of experiments involving experimental alteration of normal embryonic development in the cortex arise from the introduction of variables that may be difficult to interpret in the context of normal development. However, they provide perhaps the greatest challenge in terms of experimental design, technical skill, and interpretation.

In this series of studies, we pursue the relative role of influences of extrinsic factors in cortical development through both an observational study of normal development



(Chapter 2) and prenatal surgical alteration of it (Chapter 3). In this way, correlations between the two systems may be more easily established and correlated, and the benefits of each type of study may be obtained.



**CHAPTER 2: Correlation of  
Lateral Geniculate Nucleus Neuron Number to  
Primary Visual Cortex Neuron Number  
in Normal Macaques**





## INTRODUCTION

The findings of lateralization and asymmetry in cerebral cortex (Broca, 1861) have long interested researchers, and have been well-studied and documented, especially in the venues of language areas, structural lesions, and epilepsy (Glick, 1985). A less-explored realm within this field is that of anatomical asymmetry of bilateral structures in the cerebral cortex (Van Essen *et al.*, 1984; Galaburda *et al.*, 1991) and in the subcortical structures (Eidelberg and Galaburda, 1982). Relationships of neuron numbers, connections, and size between associated structures in bilateral systems --such as specific cortical areas and their corresponding thalamic nuclei-- displaying such asymmetry would be of particular interest in the study of cortical development. Such relationships may have functional (Williams and Rakic, 1988a), developmental (Williams and Herrup, 1988), and evolutionary (Rakic, 1988a, 1991a) implications on the role of thalamo-cortical connections in the specification of cerebral cortex.

Proponents of the theory of total specification of cerebral cortex by extrinsic factors (O'Leary, 1989) would argue that asymmetry of bilateral cortical areas in normal specimens is imposed by an asymmetry in extrinsic factors --such as thalamic input-- that affect cortical development and specification in an equally asymmetric fashion. They would predict that asymmetry in cortical innervation during development would directly affect specification of the corresponding cortical areas.

Within the protomap and radial unit hypotheses (Rakic, 1988a) one would expect the effects of asymmetries in normal cases to be limited within the prespecified boundaries of the particular cortical area. Thus, proponents of these theories would predict that asymmetries in extrinsic factors would impact on cortical patterns of connections and the resolution of input --subareal parameters-- not on areal size and boundaries.

A recent study of elimination of neurons from the LGN during development in normal rhesus monkeys showed a statistically-significant right-left asymmetry in the



population of geniculate neurons (Williams and Rakic, 1988a). The number of neurons in the right side was 8.5% higher on average than in the left side in 20 of 29 specimens examined. This asymmetry was found consistently throughout development in embryonic as well as adult material.

Is there right-left asymmetry in primary visual cortex (area 17) as well? If so, does it match that observed in the LGN? Finally, and perhaps more difficult to resolve, does LGN directly establish this asymmetry in area 17 or is size in both structures developmentally linked from the outset?

The primate visual system represents an ideal model for study of these questions. It displays remarkable fidelity of correspondence and a high degree of resolution in the connections between the retina and the lateral geniculate nucleus (LGN), and from the LGN to primary visual cortex (area 17) (Polyak, 1957; Perry *et al.*, 1984; see above). Fibers from the LGN, in fact, represent the major input into primary visual cortex. Afferent fibers from the LGN are also present in the adjacent subplate zone during neurogenesis and migration of area 17 neurons.

Given the finding of right-left asymmetry in the LGN, its strong pattern of connections with area 17, including during embryonic development, and the major role of this thalamic nucleus in carrying information to area 17, we explored the implications of differences in geniculate neuron numbers on the development and specification of primary visual cortex in normal primates. Specifically, we determined neuron numbers for LGN and ipsilateral area 17 for right and left sides in five normal macaques to examine the specific numerical relationship within subjects --comparing right and left sides in each subjects--, and the general relationship --comparing right and left sides for the population as a whole.

Determination of the relationship between neuron numbers in LGN and area 17 in normal subjects could provide insights into the role of extrinsic factors during embryonic development in the specification of cortical function and parcellation in the cerebral cortex.



## MATERIALS AND METHODS

### Subjects and Tissue Preparation

The study material was obtained from the preexisting collection of normal monkey brains prepared using cresyl violet stain at the Yale School of Medicine Section of Neurobiology. The subjects were five normal adult rhesus monkeys (*Macaca mulatta*). Their ages ranged from one to ten years.

At the time of sacrifice, the monkeys were deeply anesthetized with sodium pentobarbital, and their brains fixed by intracardiac perfusion with mixed aldehydes. The brains were blocked, embedded in celloidin, and cut in a gapless series of 35- $\mu$ m-thick coronal sections that included both right and left sides. Every 10th or 20th section was stained with cresyl violet, mounted on slides, and coverslipped.

### Estimation of LGN and Area 17 Neuron Numbers

Direct measurements of neurons in LGN and area 17 were obtained from the five normal adult monkey brains. They were obtained for right and left sides in each specimen. The process involved: 1) estimating total volumes for LGN and area 17 from serial sections; 2) obtaining sample neuron counts at random probe sites (defined below) that met predetermined criteria in LGN and area 17; 3) converting neuron counts from probe sites to total neuron estimates for each structure using volume estimate; and 4) estimating total neuron counts in each structure with 95%-confidence interval from repeated probe samplings.

**Estimation of Volumes** Outlines of the lateral geniculate nucleus (LGN) and area 17 of right and left sides of each subject were drawn from serial sections 350-700  $\mu$ m apart at specific magnifications using a *camera lucida*. Sections areas were subsequently



measured with an Apple Macintosh™ microcomputer using a Summagraphics® MacTablet™ digitizing pad and the MacMeasure 1.9™ program.

Section area in square millimeters versus rostro-caudal distance in millimeters was plotted for each section drawn using the Microsoft® Excel 2.0 spreadsheet program. The integral for the areas was obtained, yielding the volume of the structure in cubic millimeters according to Simpson's equation (Burlington, 1948). These measurements were corrected for shrinkage secondary to dehydration.

**Neuron Counts from Radial Probes in LGN and Area 17** A direct, computer-assisted, three-dimensional counting system using video-enhanced differential interference contrast (DIC) optics was employed (Williams and Rakic, 1988b) (See Figure 2-1).

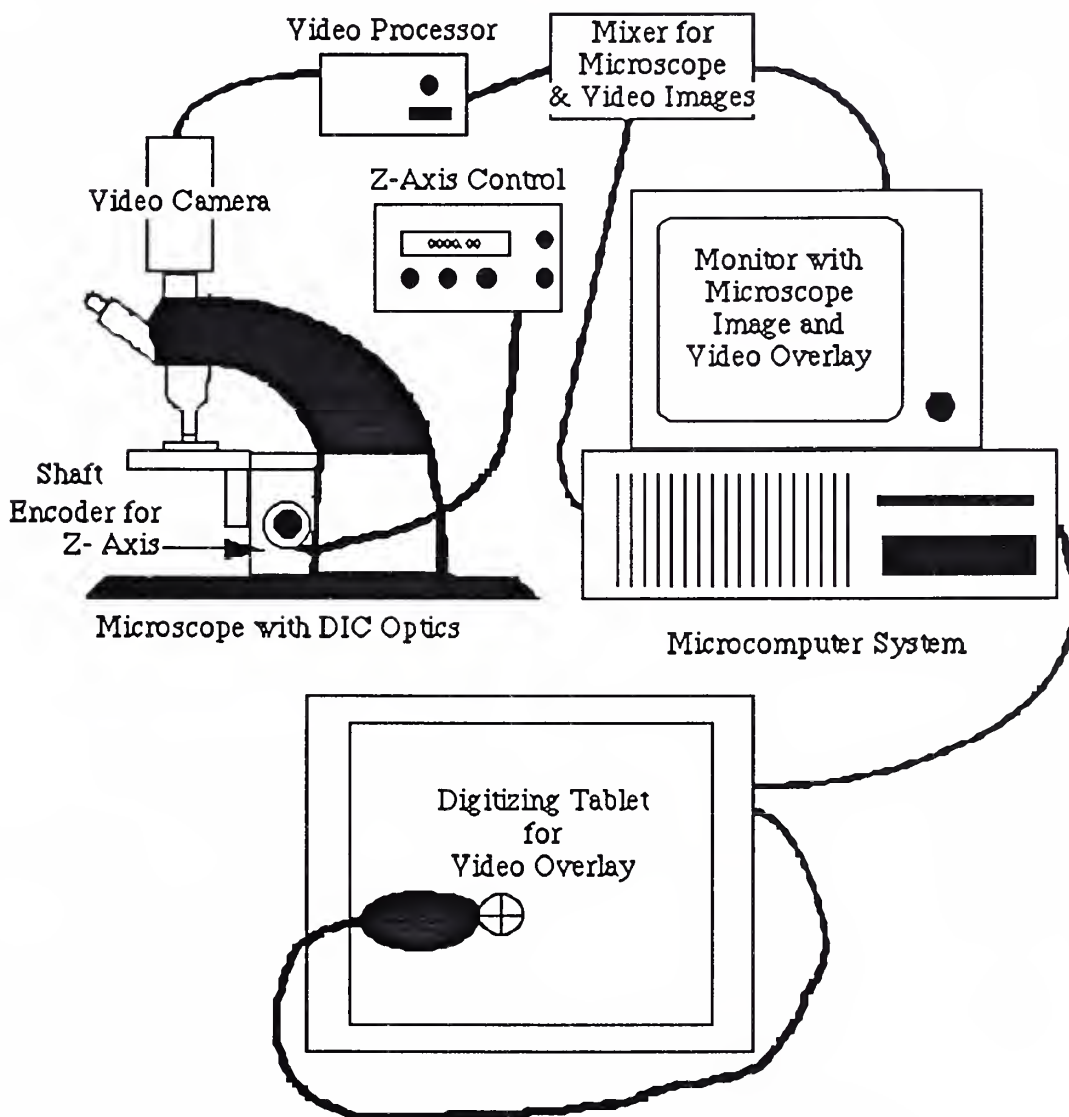
This system provides an accurate means of counting cells along radial probe sites. Each radial probe site was constructed from the end-to-end arrangement of optically-defined volumes of tissue referred to as counting boxes. Counting boxes 73  $\mu\text{m}$  high (perpendicular to LGN or cortical surface) x 63  $\mu\text{m}$  wide (parallel to LGN or cortical surface) x 20  $\mu\text{m}$  deep (perpendicular to section) were used (section thicknesses ranged from 34-40  $\mu\text{m}$ ).

Counting was performed using an IBM™-compatible microcomputer system. A computer program --"Lucida" (Robert Dolan, MIT, Cambridge, MA)-- was utilized for counting and defining probes. The image from the microscope was overlaid with the computer image from the counting system program. This program allowed for marking individual cells (one may choose from various colors and marking dot sizes) within counting boxes, and delimiting borders within the box or probe --to represent sublaminae, laminae, or cortical borders-- through a montage function. In this way, counted neurons were marked through the probe slice, thereby avoiding uncertainties in counting (whether specific neurons have or have not been counted, especially in the more densely-populated laminae), and borders were designated to aid in counting. Areas within the probe or the





**Figure 2-1:** Video-enhanced differential interference contrast (DIC) optics, computer-assisted three-dimensional counting system. Images from the DIC optics microscope and computer counting program are mixed using a video overlay system. A shaft encoder transducer provides information about the Z-axis (plane perpendicular to microscope slide surface).





entire probe were then selected to obtain total neuron counts for cortex or layers within the specified portion of cortex.

Appropriate probe sites were defined for LGN and area 17. In the LGN, appropriate probe sites crossed the six laminae of the thalamic nucleus and ran parallel to blood vessels; in visual cortex, probes were selected from opercular and calcarine sites in which layer 1 and layer 6 borders were parallel to each other, and had nearby blood vessels perpendicular to the cortical surface; probes were oriented perpendicular to the surface.

This counting method is inherently accurate: it obviates the need for correction factors; it circumvents problems caused by irregular cell shape and size, nonrandom orientation, and splitting of cells during sectioning; and it is insensitive to large variations in section thickness (Williams and Rakic, 1988b).

**Estimation of Total Neuron Counts for LGN and Area 17 from Weighted Average of Radial Probes** Total neuron numbers for LGN and area 17 were estimated from probes by utilizing the ratio of probe volume to the volume of the corresponding structure. Five radial probes were obtained for each structure. Total neuron number estimates with 95% confidence intervals of less than 10% were subsequently obtained from the weighted average of the five probes.

## RESULTS

### Lateral Geniculate Nucleus Neuron Number

The number of neurons per nucleus ranged from 1.11 million to 1.53 million with the 95%-confidence interval approximately 7% for each (see Table 2-1). The difference between subjects was statistically-significant (the difference fell outside the confidence interval) in some specimens. The neuron number for each nucleus fell into two general ranges: 1.1 - 1.3 million (2 specimens), 1.4 - 1.5 million (8 specimens) (see Figure 2-2).

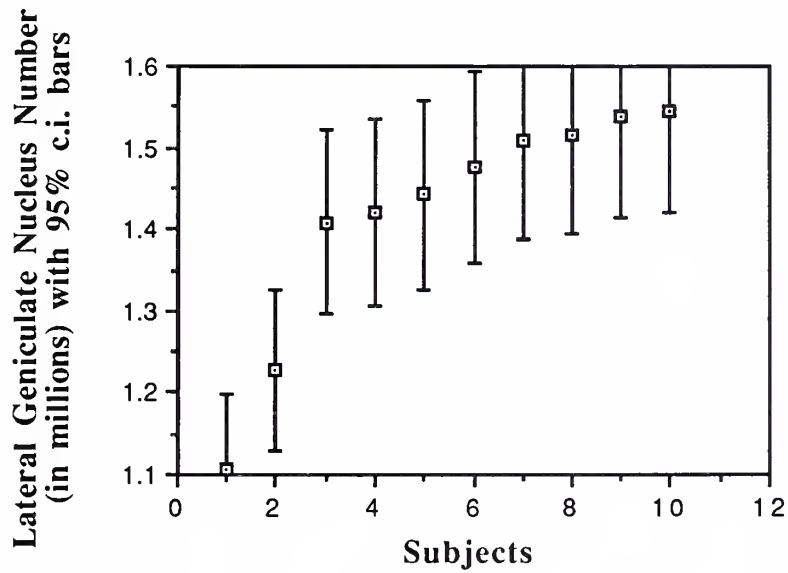


**Table 2-1:** Summary of neuron numbers for lateral geniculate nucleus (LGN) and Area 17 in millions, with 95 % confidence intervals for right and left sides of five normal adult macaques.

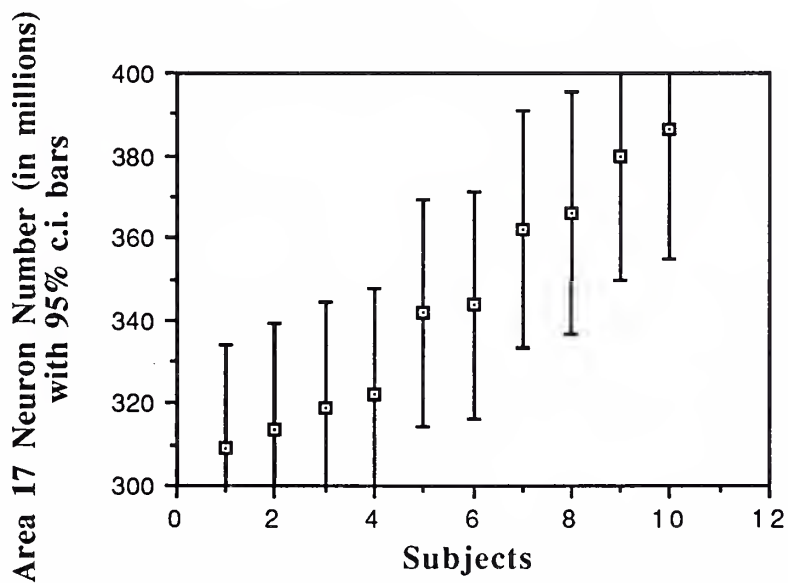
Case	Side	LGN # $\pm$ 95% c.i. (in millions)	Area 17 # $\pm$ 95% c.i. (in millions)
Celery	R	$1.307 \pm 0.102$	$314 \pm 24$
	L	$1.108 \pm 0.934$	$309 \pm 20$
Mescal	R	$1.476 \pm 0.140$	$376 \pm 16$
	L	$1.421 \pm 0.112$	$366 \pm 25$
Talisman	R	$1.538 \pm 0.115$	$324 \pm 26$
	L	$1.515 \pm 0.110$	$322 \pm 26$
Willow	R	$1.518 \pm 0.125$	$319 \pm 28$
	L	$1.544 \pm 0.145$	$342 \pm 24$
Zulu	R	$1.428 \pm 0.074$	$380 \pm 33$
	L	$1.408 \pm 0.125$	$362 \pm 31$



**Figure 2-2:** Plot of lateral geniculate nucleus number (in millions) with 95%-confidence interval. Specimens were arranged in increasing number of LGN neurons.



**Figure 2-3:** Plot of area 17 neuron number (in millions) with 95%-confidence-interval. Specimens were arranged in increasing number of area 17 neurons.







In general, the right-left difference in each subject was within the 95% confidence interval, save for one specimen --Celery-- where the right-left difference was clearly outside the confidence interval. Interestingly, the total neuron estimate for the right LGN was greater than that of the corresponding left nucleus in four of the five specimens, although again, it was statistically-significant in only one of the specimens.

### **Area 17 Neuron Number**

The number of neurons per hemisphere in area 17 ranged from 309 million to 380 million with the 95%-confidence interval approximately 8% (see Table 2-1). The difference between subjects was statistically-significant (the difference fell outside the confidence interval) in some specimens. The neuron number for each hemisphere fell into two general ranges: 300-320 million (4 specimens), 350-380 million (4 specimens). Two specimens fell in the 340 million range, which could not be separated from either of the previous groups with a 95%-degree of confidence (see Figure 2-3).

In general, the right-left difference within each subject was within the 95% confidence interval. Interestingly, the total neuron estimate for the right area 17 was greater than that of the corresponding left primary visual cortex in four of the five specimens.

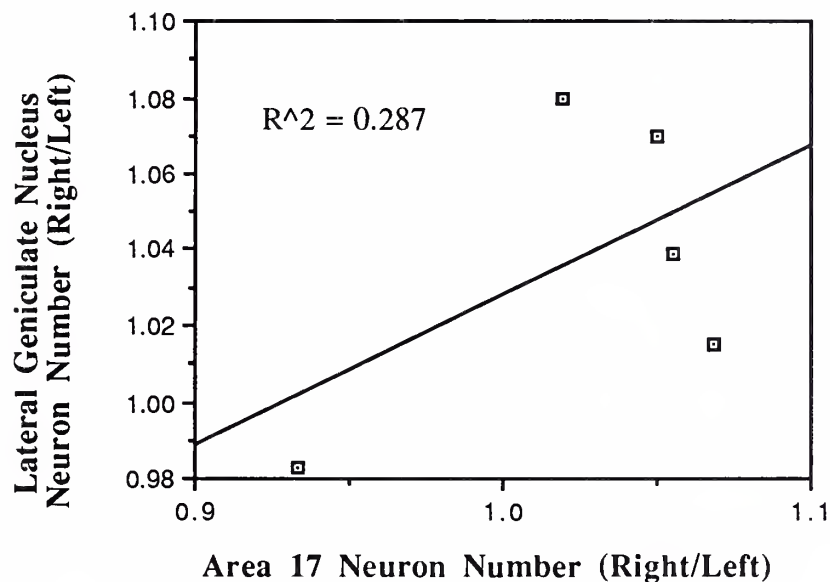
### **Lateral Geniculate Nucleus Neuron Number versus Area 17 Neuron Number**

**Relationship with respect to right-left asymmetry** A plot of LGN versus area 17 neuron numbers was constructed using the ratio of neurons on the right side:neurons on the left side (see Figure 2-4). Again, the correlation between the two was weak (correlation coefficient = 0.536,  $r^2 = 0.287$ ), but LGN and area 17 coincided by specimen as being greater than one or less than one in five of five specimens.

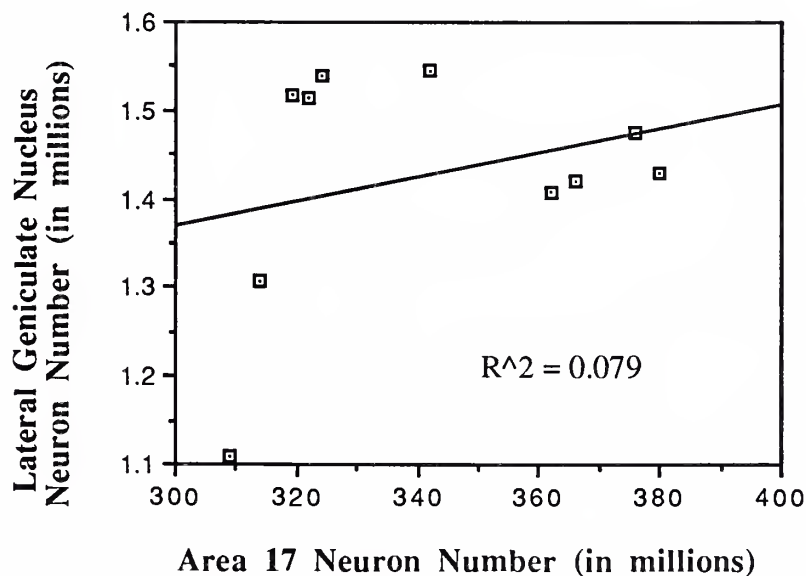
**Relationship for population as a whole** The number of neurons in each LGN was compared with that of the ipsilateral area 17 in each specimen (see Table 2-1). Unfortunately, both LGN and area 17 neuron numbers were clustered (see above) and,



**Figure 2-4:** Plot of right:left ratios of lateral geniculate nucleus neuron numbers versus that of Area 17 neuron numbers for each animal.  $R^2$  statistic for regression line is shown.



**Figure 2-5:** Plot of lateral geniculate nucleus neuron number (in millions) versus Area 17 neuron number (in millions) for each side of each animal.  $R^2$  statistic for regression line is shown.





thus, little resolution between values was possible despite 95%-confidence intervals within 10% of the values.

A plot of LGN neuron number versus area 17 neuron number for all specimens was constructed (see Figure 2-5). There was a general correlation between LGN and area 17 neuron numbers, but this was not a strong one (correlation coefficient=0.28;  $r^2=0.079$ ).

Interestingly, within each specimen, LGN and Area 17 neuron numbers were of the same order. Also, a pattern right-left asymmetry seemed to hold where the same four of the five specimens had greater neuron numbers in both LGN and area 17 in the right side. However, it must be pointed out that right-left differences were within 95%-confidence intervals save for one specimen in LGN and one specimen in area 17 (different subjects).

## DISCUSSION

The role of extrinsic influences such as thalamic input in specification of cortical areas is a intensely pursued question in developmental neurobiology (Rakic, 1988a; O'Leary 1989). Investigators have approached this question by either rerouting peripheral retinal input to an inappropriate thalamic nucleus and cortical area and testing processing in nonvisual cortical areas (Frost and Metin, 1987; Sur *et al.*, 1988) or heterotopic transplantation of embryonic cortical cells (McConnell, 1985; O'Leary and Stanfield, 1989).

In this study we sought to explore this question from the perspective of normal development in the macaque primary visual cortex. Critical to the selection of this system as the experimental model were the following criteria: 1) a cortical area that is closely linked with a specific thalamic nucleus; and 2) a report demonstrating right-left asymmetry in the LGN of macaques (Williams and Rakic, 1988a). We investigated the effects of normal, asymmetric thalamic input from the LGN in the specification of neuron number in



area 17 within each subject --to search for right-left asymmetry-- , and for the population as a whole --to search for a general trend in LGN:area 17 ratios.

Five normal adults were examined for these parameters. Results of counting pointed at little variability in LGN and area 17 neuron numbers (see Table 2-1). In the LGN, all measurements but one fell within a similar confidence interval, despite error parameters within 8% of the estimate. The values for neuron numbers in the LGN of the ten nuclei measured were clustered about two discreet groups (see Figure 2-2). In area 17, a similar situation was noted despite, again, confidence intervals within 7% of the estimate. The values were clustered in two discreet groups and one in the middle bridging these two (see Figure 2-3).

There was a consistent finding of right-left asymmetry both in LGN and area 17 with four of five specimens having greater neuron numbers on the right side than the left (see Table 2-1). However, while the LGN difference was not statistically-significant in three subjects, in only one subject --a different one than those in the previous statement-- was the area 17 right-left differential statistically significant. Again, this lack of statistically-significant difference was noted despite confidence intervals within 7-8% of the estimated neuron numbers.

One of the factors explored in this study related to previous observations of right:left asymmetry in the LGN (Williams and Rakic, 1988). When this relationship was compared within subjects with respect to right-left asymmetry, the trend was present but, again, the correlation was rather weak (see Figure 2-4). It is important to note, however, that the right:left ratios corresponded for all five specimens; four of five fell in the quadrant corresponding to LGN:area 17 right:left ratios greater than one (both neuron numbers were greater in the right than in the left), and the remaining specimen was fell in the quadrant corresponding to right:left ratios less than one (neuron numbers in the left LGN and area 17 were greater than ones on the right).





Thus, from these results, it appears that neuron number in area 17 does not display the same degree of asymmetry as that observed in the LGN. Such findings may be attributed to stability of cortical number related to area-specific intrinsic properties, or perhaps due to equalizing factors imposed by extrinsic factors other than the LGN innervating area 17.

The correlation between LGN neuron number and area 17 neuron number for the population as a whole was weak (see Figure 2-5). Stronger evidence is found in one specific relationship: one specimen --Willow-- had an LGN neuron numbers that fell in the statistically-significant high cluster for the series, while the area 17 neuron numbers fell in the statistically-significantly low cluster.

It may be implied from these results that there is not a gross relationship between area 17 neuron numbers and those of the LGN in adults. That, indeed, the relationship between total neuron numbers LGN and area 17 neuron numbers is not a direct one and other factors may be involved in determining final neuron number in primary visual cortex in adult primates. For example, input from other cortical areas may have a greater impact on final neuron number than that of the direct relationship with the LGN, and, therefore, direct effects on area 17 neuron numbers may be obscured.

Another possibility is that the relationship between LGN and area 17 may be more apparent in the prenatal or perinatal periods. Other factors, such as selective cell death (Williams and Herrup, 1986) may act to equalize differences in neuron numbers related to LGN influence; or following a direct effect of the LGN, other extrinsic factors act to equalize differences. Indeed a greater degree of cell death occurs in the supragranular layers between embryonic days 118-128 (Williams *et al.*, 1987). Thus, LGN influences may be most apparent prenatally or perinatally before other factors act to modify final neuron numbers in area 17.

On the other hand, one may argue that there may not be as much functional asymmetry in LGN input as is at first apparent. Total neuron number in the LGN may not



represent a one-to-one correspondence with synaptic input to area 17, which would be a more precise measurement of thalamic input. In this case, anatomical asymmetries may not necessarily be functional asymmetries in terms of input. Perhaps investigating this issue by examining the correlation of total neuron number with other parameters such as density of projections to cortex and terminal synapses within various layers may resolve this question.

The possibility of alpha error is also a valid consideration. The failure to find difference in this study may relate to its design or methods. Possible sources of alpha error include the study's power with respect to number of subjects, and the counting method's sensitivity. The ten specimens were obtained from five subjects prepared by histological methods in which both sides of LGN and area 17 were included; this was intended to allow for the second arm of the study: exploring right-left differences within subjects.

In terms of the sensitivity of the counting method, this method provides perhaps the most reliable available way of determining neuronal populations (Williams and Rakic, 1988a). This was evidenced by average confidence intervals on the order 7-8%.

Thus, there is apparently more asymmetry in LGN than in area 17. This would imply perhaps a clearer separation between the relative roles of intrinsic factors, in which a more global effect on cortical specification is hypothesized, versus those of extrinsic factors, which are thought to have a role at the sublayer or subareal level.

The influences in development from asymmetric LGN input to area 17 appear to be too subtle in normal adult monkeys to definitively resolve the questions set forth in the introduction. A model in which more obvious differences in afferent input to area 17 might bring out these differences in a clearer fashion. Such a model, in which LGN input to area 17 is diminished by prenatal surgical manipulations, is applied to study these questions in the following chapter.



**CHAPTER 3: Effects of Prenatal Reduction of  
Geniculocortical Input on  
Specification of Primary Visual Cortex**



## INTRODUCTION

The role of extrinsic factors such as thalamo-cortical interactions in the specification of areal borders and cytoarchitecture during development of the cerebral cortex has been an actively-pursued question among developmental neurobiologists (Rakic, 1988; O'Leary, 1989; Jones, 1990; Windrem and Finlay, 1991). The central issue relates to whether migrating neurons leaving the ventricular zone are completely pluripotential and dependent on extrinsic factors for determination of area-specific neuronal phenotype; or whether these neurons carry a level of area-specific commitment that may be fine-tuned or altered by extrinsic factors (the protomap hypothesis).

This study tested the role of extrinsic factors in the specification of area borders and cytoarchitecture in the cerebral cortex by means of surgical reduction of thalamic neuron number during embryonic development. The model utilized was the macaque visual system. This system was apt for this study for a variety of reasons: 1) there is great fidelity in the primate visual system with over 90% of retinal input going to LGN (Polyak, 1957; and Perry *et al.*, 1984), and over 90% of LGN input sent to area 17 (Norden and Kaas, 1978; Pasik *et al.*, 1986); 2) the LGN serves as the major input to area 17 ; in turn, the LGN is a major target of cortical afferents from area 17 (Polyak, 1957); 3) the characteristic cytoarchitecture of normal primate area 17 allows for clear demarcation of area boundaries (Brodmann, 1905; Polyak, 1957); 3) the layer-specific patterns of neuronal types and connections in primate area 17 are wellcharacterized (reviewed in Valverde, 1985); 4) the timing of events during the development of primary visual cortex in primates has been precisely delineated (Rakic, 1974, 1977); 5) there is a strong anatomic correlation between the macaque's visual system and that of humans; and 6) the technical means of reducing lateal geniculate nucleus number prenatally is available (Rakic, 1988a).

Reduction of LGN input to area 17 was performed at specific stages of cortical development. Three events in the development of LGN and area 17 were targetted in these





experimental animals: 1) LGN neurons are generated during embryonic days 36 and 43 (E36-E43), 2) LGN fibers to visual cortex remain in the subplate zone until invasion of primary visual cortex layers IVA and IVC occurs during E110-E120, and 3) genesis of cortical neurons in area 17 occurs during E43-102, with those destined for layer IV --which receives all LGN input-- generated during E70-E85 (Rakic, 1977).

The two earliest binocular enucleations were performed at embryonic days 59 and 65 (E59 and E65), at which time all LGN neurons have been generated, LGN efferent fibers lie in the subplate zone within the route of migrating cortical neurons, and before neurons destined to populate layer IV in area 17 have been generated (Rakic, 1977). Two other subjects (E81 and E90) were enucleated during generation of neurons destined for layer IV in area 17, respectively; LGN fibers were still in subplate zone at time of enucleation. The final specimen (E120) was enucleated after all area 17 neurons have been generated and LGN fibers have penetrated the cortical plate.

The LGN and area 17 were examined with regard to size, gross cytoarchitecture, and total neuron number. In area 17, the cases were further-characterized by analysis of individual cortical layer thickness and neuron density. A novel cytoarchitectonic area -- termed area X-- was found within area 17 and between 17-18 borders in two of the cases; it was also characterized for these parameters.

## MATERIALS AND METHODS

### Subjects and Tissue Preparation

The study material was obtained from the preexisting histological collection of the Yale School of Medicine Section of Neurobiology. The subjects were five experimental rhesus monkeys (*Macaca mulatta*), and five normal controls. Two of the specimens have been described in an initial publication on this subject (Rakic, Suñer, and Williams, 1991), and three others are added to the series. All experimental animals underwent bilateral optic



nerve resections during embryonic development, as described below, and were subsequently sacrificed between the ages of two months and three years.

Pregnant females at various gestational ages were selected: Embryonic Day 59 (E59), E67, E81, E90, and E120. In preparation for the procedure they were intubated endotracheally, and induced with halothane anesthesia. Hysterotomies were performed via midline abdominal incisions and the embryos were exposed. Both eyes were removed from the embryos, and they were returned to the uterus. The incisions were closed, and the mothers returned to their cages. The healthy offspring were then delivered at term (E165).

At the time of sacrifice, the monkeys were deeply anesthetized with sodium pentobarbital, and their brains fixed by perfusion through the heart with mixed aldehydes. Generally, one hemisphere was available for cytology, while the other was used for other studies. The brains were blocked, embedded in celloidin, and cut in 35- $\mu$ m-thick coronal sections. They were stained with cresyl violet.

### **Estimation of LGN and Area 17 Volumes and Neuron Numbers**

Direct measurements of neurons in LGN and area 17 were obtained from the five normal adult monkey brains. They were obtained for right and left sides in each specimen. The process involved: 1) estimating total volumes for LGN and area 17 from serial sections; 2) obtaining sample neuron counts at random probe sites (defined below) that met predetermined criteria in LGN and area 17; 3) converting neuron counts from probe sites to total neuron estimates for each structure using volume estimate; and 4) estimating total neuron counts in each structure with 95%-confidence interval from repeated probe samplings.

**Estimation of Volumes** Outlines of the lateral geniculate nucleus and area 17 of each subject were drawn from serial sections 350-700  $\mu$ m apart at specific magnifications using a *camera lucida*. Sections areas were subsequently measured with an Apple



Macintosh™ microcomputer using a Summagraphics® MacTablet™ digitizing pad and the MacMeasure 1.9™ program.

Section area in square millimeters versus rostro-caudal distance in millimeters was plotted for each section drawn using the Microsoft® Excel 2.0 spreadsheet program. The integral for the areas was obtained, yielding the volume of the structure in cubic millimeters according to Simpson's equation (Burington, 1948). These measurements were corrected for shrinkage secondary to dehydration.

**Neuron Counts from Radial Probes in LGN and Area 17** A direct, computer-assisted, three-dimensional counting system using video-enhanced differential interference contrast (DIC) optics was employed (Williams and Rakic, 1988b).

This system provides an accurate means of counting cells along radial probe sites. Each radial probe site was constructed from the end-to-end arrangement of optically-defined volumes of tissue referred to as counting boxes. Counting boxes 73  $\mu\text{m}$  high (perpendicular to LGN or cortical surface) x 63  $\mu\text{m}$  wide (parallel to LGN or cortical surface) x 20  $\mu\text{m}$  deep (perpendicular to section) were used (section thicknesses ranged from 34-40  $\mu\text{m}$ ).

Counting was performed using an IBM™-compatible microcomputer system. A computer program -- "Lucida" (Robert Dolan, MIT, Cambridge, MA)-- was utilized for counting and defining probes. The image from the microscope was overlayed with the computer image from the counting system program. This program allowed for marking individual cells (one may choose from various colors and marking dot sizes) within counting boxes, and delimiting borders within the box or probe --to represent sublaminae, laminae, or cortical borders-- through a montage function. In this way, counted neurons were marked through the probe slice, thereby avoiding uncertainties in counting (whether specific neurons have or have not been counted, especially in the more densely-populated laminae), and borders were designated to aid in counting. Areas within the probe or the



entire probe were then selected to obtain total neuron counts for cortex or layers within the selected portion of cortex.

Appropriate probe sites were defined for LGN and area 17. In the LGN, appropriate probe sites crossed the six laminae of the thalamic nucleus and ran parallel to blood vessels; in visual cortex, probes were selected from opercular and calcarine sites in which layer 1 and layer 6 borders were parallel to each other, and had nearby blood vessels perpendicular to the cortical surface; probes were oriented perpendicular to the surface.

Borders for laminae within area 17 were delimited within radial probe sites using criteria described by O'Kusky and Colonnier (O'Kusky and Colonnier, 1982). Layers delimited included: I, II and III, IVA, IVB, IVC $\alpha$ , IVC $\beta$ , V, and VI. This computer-aided method allowed for calculation of thicknesses and neuron densities for each layer and sublayer.

This counting method is inherently accurate: it obviates the need for correction factors; it circumvents problems caused by irregular cell shape and size, nonrandom orientation, and splitting of cells during sectioning; and it is insensitive to large variations in section thickness (Williams and Rakic, 1988b).

**Estimation of Total Neuron Counts for LGN and Area 17 from Weighted Average of Radial Probes** Total neuron numbers for LGN and area 17 were estimated from each probe by utilizing the ratio of probe volume to the volume of the corresponding structure. Five radial probes were obtained for each structure. Total neuron number estimates with 95% confidence intervals of less than 10% were subsequently obtained from the weighted average of the five probes. Similarly, densities for each layer or sublayer were calculated.





## RESULTS

### **Lateral Geniculate Nucleus Volumes, Lamination, and Neuron Number**

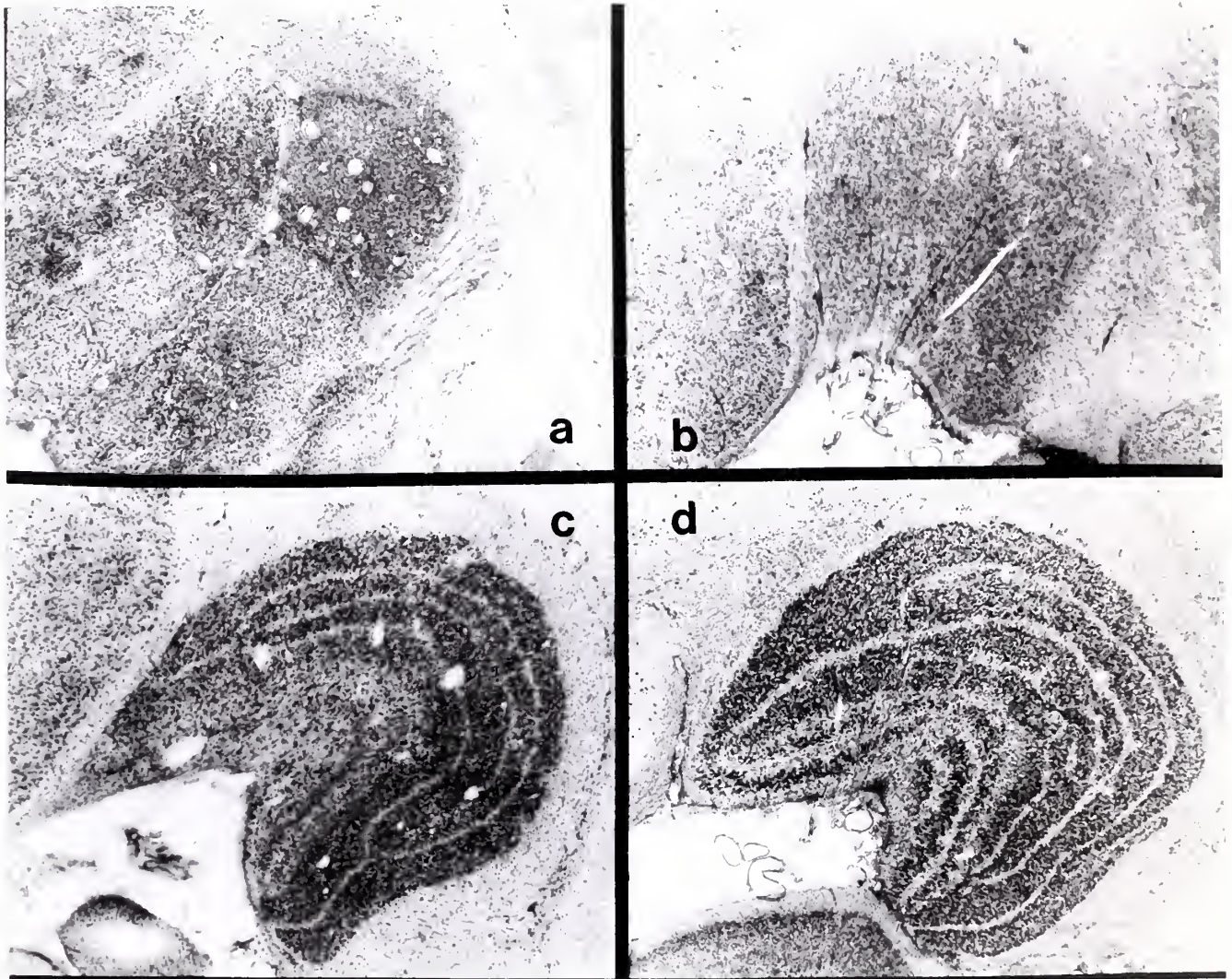
A continuum in the lamination, volume, and neuron number of the LGN was observed in the experimental animals when compared to controls. On gross examination of sections, there was a glaring absence of normal LGN lamination and distinction between magnocellular and parvocellular neurons in all experimental cases, save for the E120 specimen (see Figure 3-1). In the two earliest enucleations, E59 and E67, the LGN was gliotic and diffusely populated. The E81 and E90 cases were, again, lacking in lamination pattern, although more densely populated than the earlier cases. The E120 specimen, on the other hand, displayed an anatomically-normal pattern of lamination, including separation of magnocellular and parvocellular populations.

The data for volume and total neuron number revealed a progression from the earliest-enucleated to the latest-enucleated cases (see Table 3-1). In the E59 and E67 cases, LGN volume was 40-50% compared to controls, with total neuron numbers 30-35% of normal values observed. These numbers increased in the E81 and E90 cases to 85%-90% of normal volume and 60-80% of the normal neuron population; interestingly, despite approximation of normal values, there was absence of lamination in these cases. The latest enucleate, E120, showed LGN volume and neuron populations within normal limits, although the magnocellular layers were, on gross examination, less populated than in normal animals (see Figure 3-1).

These results demonstrate statistically-significant differences in the LGN populations, not only at the anatomical level of neuron numbers, but also implicitly at the functional level in terms of lamination pattern and subdivision into magnocellular and parvocellular populations.



**Figure 3-1:** Coronal sections showing greatest diameter of lateral geniculate nuclei (LGN) in macaque monkeys undergoing binocular enucleation and in controls. Representative cases show: a) binocular enucleation on embryonic day 59 (E59), b) E90, and E120; d) is a normal control. LGN's are photographed at same level of magnification. Cresyl violet-stained 35  $\mu$ m-thick sections.





## Area 17 Surface Area, Cytoarchitecture, and Neuron Number

Surface area measurements for area 17 in cases E59, E67, and E81 were significantly less than those observed in normal controls (see Table 3-1). A consistent pattern of greater reduction with earlier date of enucleation was observed, with the earliest enucleate --E59-- retaining 39% of normal and the E81 specimen 87%.

Gross examination of area 17 in experimental cases revealed normal lamination patterns including sharp borders characterized by the abrupt termination of the pale stria of Gennari, unique to primate primary visual cortex and corresponding to layer IVB (Brodmann, 1905; Polyak, 1957). Abnormal patterns of gyrations were noted most prominently in the E59 and E67 cases, but were also present to a lesser degree in E81 and E90 cases.

Gross cytoarchitectonic aberrations were noted in the E67, E81, and E90 cases. In the E67 specimen, a small island of area 17 was noted in the operculum through three consecutive sections (see Figure 3-2). There appeared to be area 18 or an area 18-like area between it and the continuous opercular portion of area 17; its total surface area was approximately one square millimeter.

In E81, a cytoarchitectonic area different from areas 17 and 18 was noted within area 17 (see Figure 3-3) and between area 17 and area 18 borders (see Figure 3-4). These relationships are represented in a two-dimensional reconstruction of the visual cortex (see Figure 3-5). Interestingly, the summation of surface areas of portions of this novel cytoarchitectonic area --here termed area X-- represented the difference between area 17 surface area in E81 and that in normal controls (see Table 3-1).

The cytoarchitectonic pattern of area X was readily distinguishable from those of area 17 and area 18 (see Figure 3-6). Area X was characterized at the gross level by: 1) cortical thickness similar to that of Area 17 (Area 18 is thinner, as is the remainder of the cerebral cortex); 2) diffuse, homogeneous layer IV that, unlike area 17 did not show sublaminae IVA, IVB (stria of Gennari), IVC $\alpha$ , and IVC $\beta$ , and unlike area 18 was not as





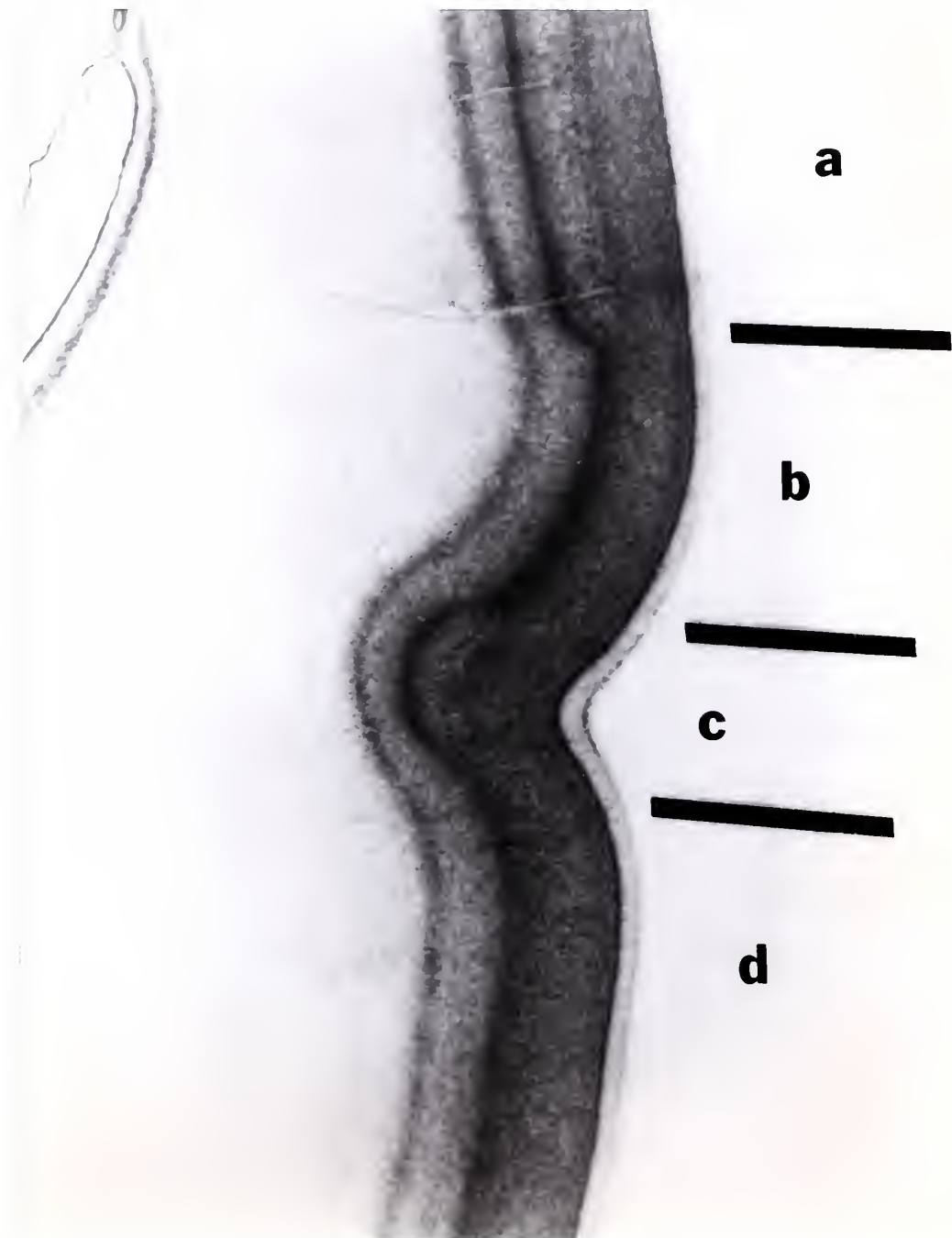
**Table 3-1:** Comparison of size and neuron number parameters for Area 17 (A17), Area X (AX), and lateral geniculate nucleus (LGN) in experimental and control cases. For A17 and AX, surface area (S.A.) is in square millimeters; for LGN, Volume (Vol.) is in cubic millimeters. Total neuron number (Neuron #) is in millions  $\pm$  95% confidence interval. “E#” denotes embryonic date in days at which binocular enucleation was performed in experimental cases. “Cntl” denotes average of controls.

<u>Case</u>	<u>A17</u>		<u>AX</u>		<u>LGN</u>	
	<u>S.A.</u>	<u>Neuron #</u>	<u>S.A.</u>	<u>Neuron #</u>	<u>Vol.</u>	<u>Neuron #</u>
<b>E59</b>	253	159 $\pm$ 14	0	0	9	0.43 $\pm$ 0.03
<b>E67</b>	281	178 $\pm$ 30	0	0	10	0.52 $\pm$ 0.07
<b>E81</b>	566	247 $\pm$ 13	73	24 $\pm$ 3	17	0.83 $\pm$ 0.05
<b>E90</b>	661	302 $\pm$ 18	13	4.2 $\pm$ 0.1	19	1.09 $\pm$ 0.08
<b>E120</b>	652	347 $\pm$ 34	0	0	21	1.41 $\pm$ 0.10
<b>Cntl</b>	654	310 $\pm$ 28	0	0	20	1.34 $\pm$ 0.08



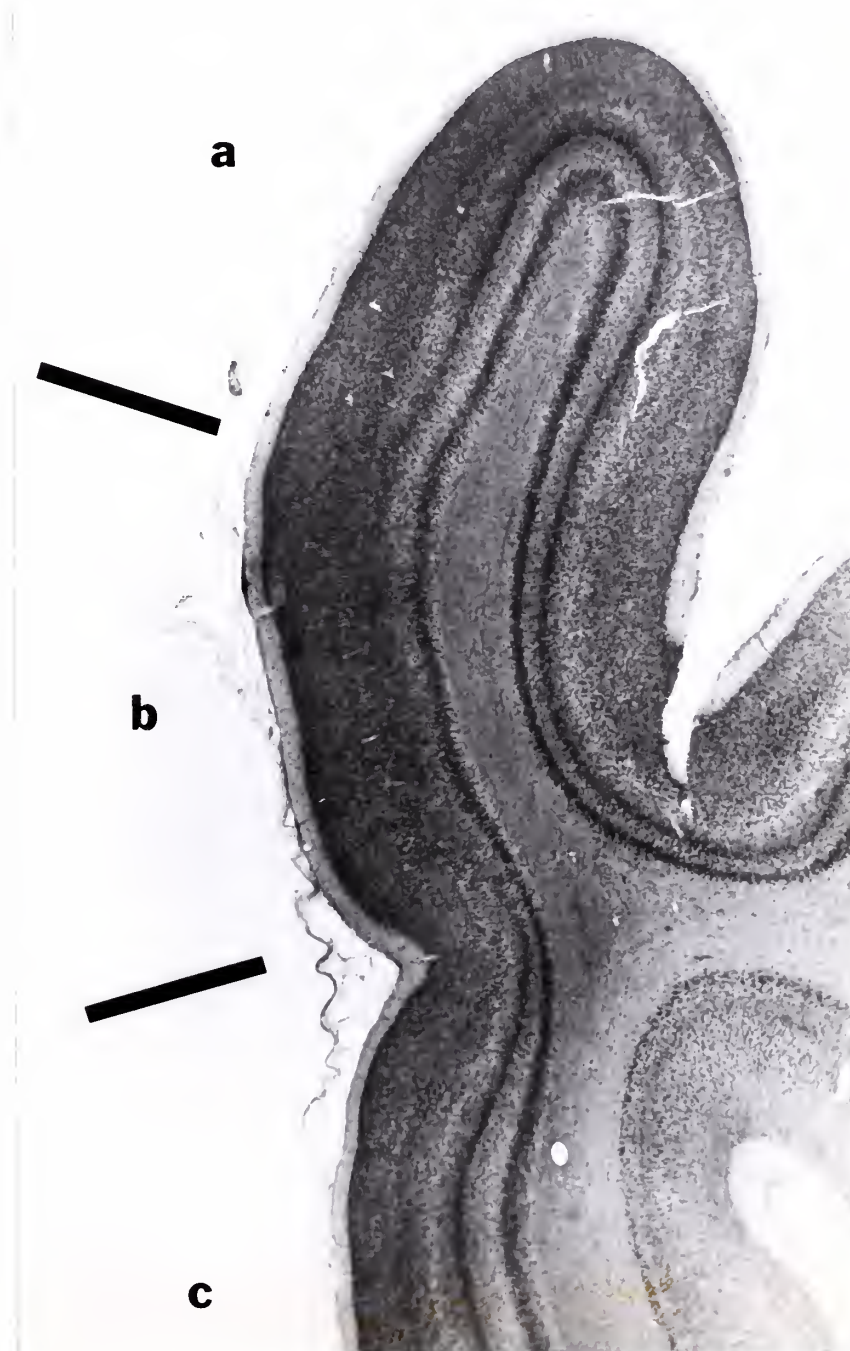


**Figure 3-2:** Coronal section across lateral surface of occipital cortex in macaque monkey binocularly enucleated at E67. This figure shows an island of area 17 discontinuous with the remainder of area 17. Borders between cytoarchitectonic areas are denoted by arrows. a) represents normal area 17 in continuity with remainder of primary visual cortex; b) denotes region whose cytoarchitecture resembles that of area 18; c) denotes region of area 17 discontinuous with the remainder of primary visual cortex (note wider cortex and pale band of layer IVb, or stria of Gennari; and d) denotes region whose cytoarchitecture resembles that of area 18. Cresyl violet-stained 35  $\mu\text{m}$ -thick section.



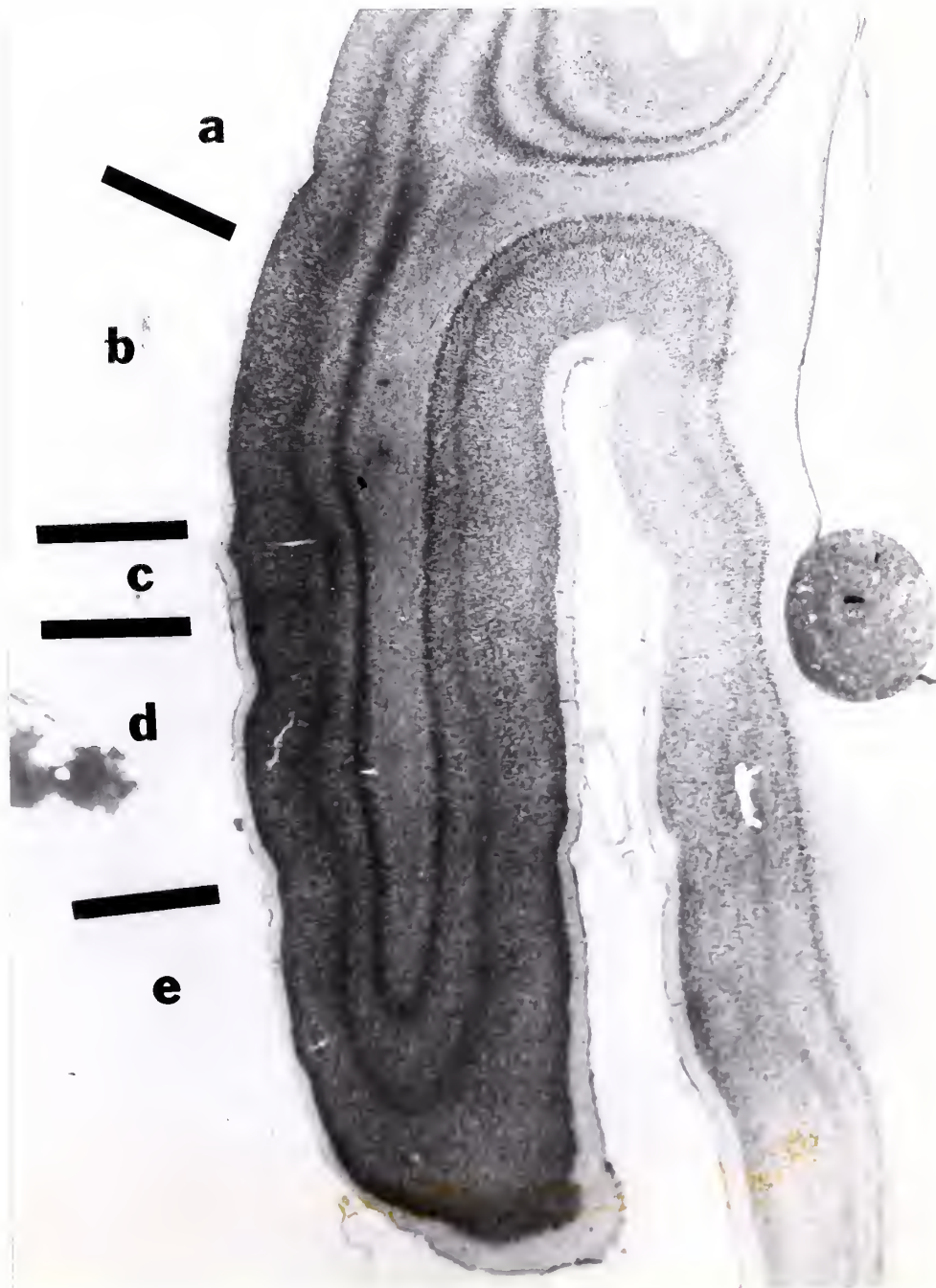


**Figure 3-3:** Coronal section across lateral surface of occipital cortex in macaque monkey binocularly enucleated at E81. This figure shows the presence of a novel cytoarchitectonic area, here termed area X, within normal area 17. Borders between cytoarchitectonic areas are denoted by arrows. a) denotes region of normal area 17; b) denotes region of area X, whose cytoarchitectonic features differ from those of areas 17 and 18; c) denotes region of normal area 17. Cresyl violet-stained 35  $\mu\text{m}$ -thick section.





**Figure 3-4:** Coronal section across lateral surface of occipital cortex in macaque monkey binocularly enucleated at E81. This figure shows the presence of a novel cytoarchitectonic area, here termed area X, within the borders of area 17 and between the borders of areas 17 and 18. Borders between cytoarchitectonic areas are denoted by arrows. a) denotes region of normal area 17; b) denotes region of area X, whose cytoarchitectonic features differ from those of areas 17 and 18; c) denotes region of normal area 17; d) denotes region of area X; e) denotes normal area 18. Cresyl violet-stained 35  $\mu$ m-thick section.



1. 1000

2. 1000

3. 1000

4. 1000

5. 1000

6. 1000

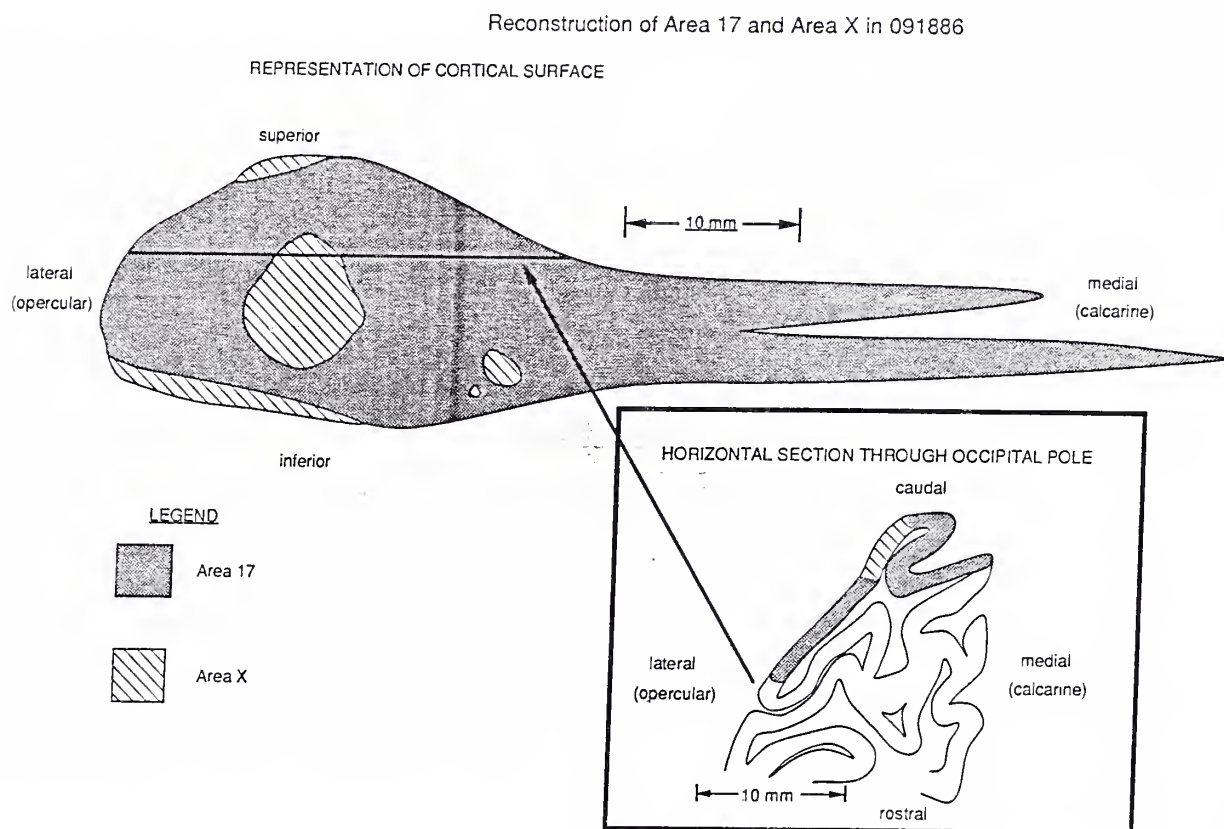
7. 1000

8. 1000

9. 1000



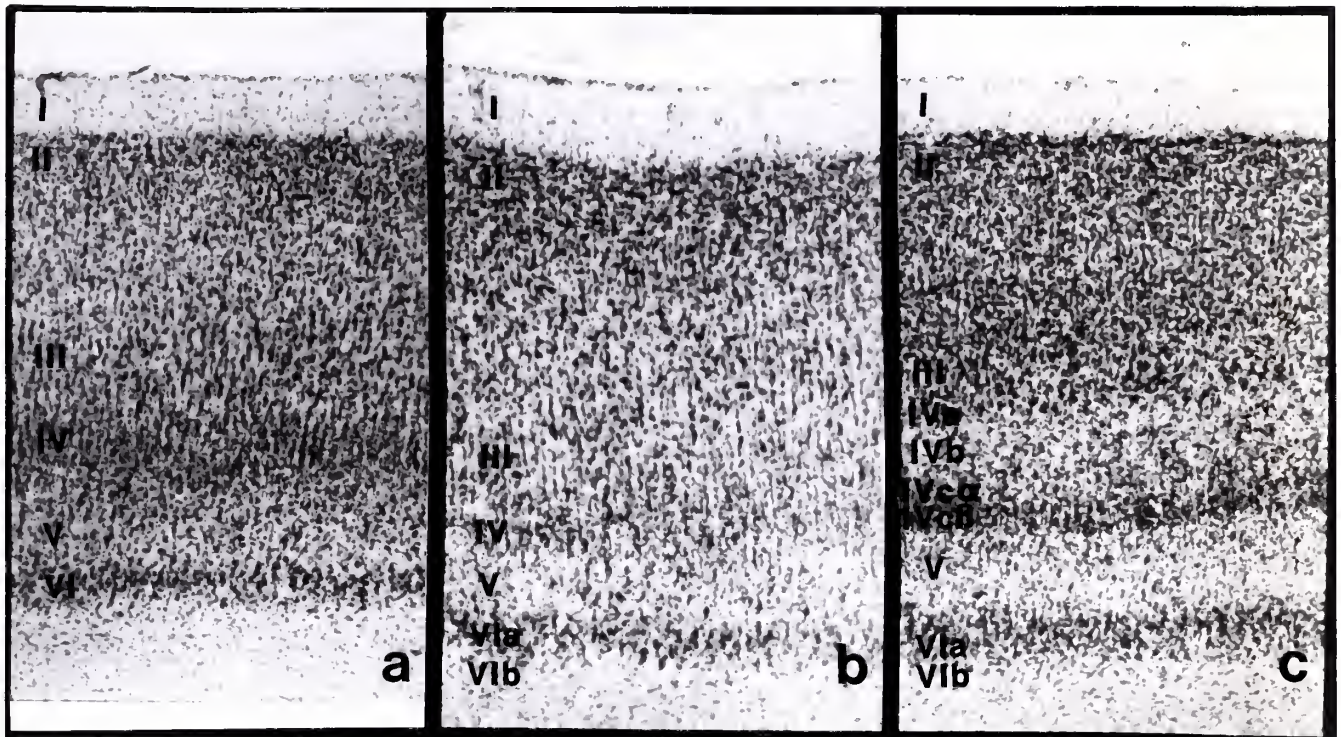
**Figure 3-5:** Computer-aided reconstruction of the visual cortex from serial sections of the occipital lobe of monkey binocularly enucleated at E81 (after Van Essen and Maunsell, 1980). The shaded surface represents the extent of cytoarchitecturally-normal area 17; the hatched surface represents the extent of area X. The horizontal line across the reconstructed area denotes the position of the section outlined in the lower right side.







**Figure 3-6:** Representative examples of the cytoarchitectonic patterns in a) area 18, b) area X, and c) area 17 in a macaque monkey binocularly enucleated at embryonic day 81 (E81). Designation of area 17 and area 18 cytoarchitectonic parameters are after Brodmann (1905). Area X has not been previously described. Cresyl violet-stained 35  $\mu\text{m}$ -thick sections.





confined; and 3) layers V, VIA, and VIB similar to those of area 17 (Area 18 has a homogeneous layer VI) (Brodmann, 1905; Polyak, 1957; O'Kusky and Colonnier, 1982).

This novel cytoarchitectonic area was also observed in the E90 specimen. However, in this specimen it was present only between area 17 and area 18 borders. Furthermore, the surface area of area X in E90 was much less than in the E81 specimen (see Table 3-1).

Examination of total neuron numbers and laminar distribution of neuronal populations revealed: 1) an identical trend for total neuron number as that observed for surface area; 2) indistinguishable layer-specific neuronal densities for area 17 in the experimental cases and normal controls; and a unique lamination pattern for the novel cytoarchitectonic area --now termed area X-- found in cases E81 and E90.

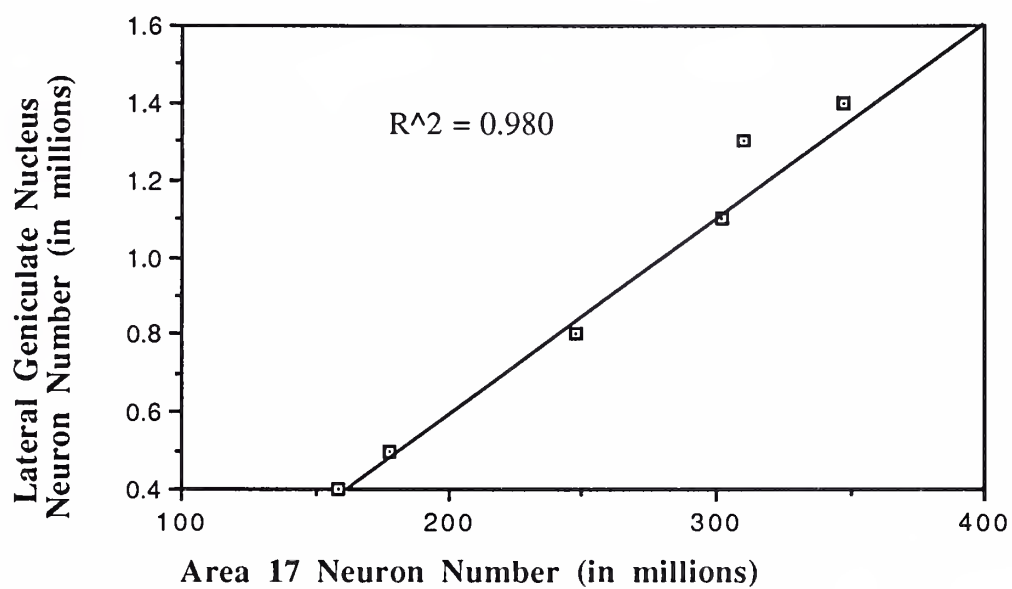
Reductions in total neuron number consistent with the surface area reductions were observed in the early enucleates (see Table 3-1). In E59, the surface area was 39% of that obtained for normal controls, while the total neuron number was approximately 50% of normal controls. The E67 specimen showed slightly increased parameters, but both were still less than 60% that of normals. The E81 specimen had a surface area 87% and total neuron number 80% that observed in controls. Again, along the same lines as with surface area, if the complement of neurons from area X were summed to those of area 17, the figure would approximate that of normal controls. Both E90 and E120 cases, on the other hand had surface area and total neuron measurements within the confidence intervals of those measured in normal controls.

A strong correlation between total neuron number in LGN and area 17 was observed ( $r^2 = 0.98$ ) (see Figure 3-7).

Examination of layer-specific neuron densities in area 17 of enucleates revealed consistent with that of area 17 in normal controls (see Table 3-2). This adds weight to this model in terms of fidelity in laminar specification of area 17 despite reduced surface area, and demonstrates a consistent process in lamination despite alteration in thalamic inputs at



**Figure 3-7:** Plot of lateral geniculate nucleus neuron number (in millions) versus Area 17 neuron number (in millions) for each experimental animal and for the average of control animals.  $R^2$  value for regression line is shown.





various junctures during which position of thalamic afferents and stage of cortical neuron migration and interactions are changing (Rakic, 1974).

Layer-specific analysis of area X and area 18 was performed for comparison with area 17 (see Table 3-3). This layer-by-layer comparison highlighted and further characterized area differences that were observed grossly. The thickness of layer 2,3 in area X was comparable to that of area 18, which is significantly thicker than that of area 17; Differences in neuron densities between the three were not statistically-significant.

Layers IVA, IVB, and  $IVC\alpha$ , and  $IVC\beta$  in area 17 were combined for ease in comparison with the homogeneous layer IV in both area X and area 18. Layer IV, of course, plays a key role in area 17 as it is the target site for afferent fibers from the LGN, and its borders contain 45-50% of the neurons within a radial probe through area 17 (O'Kusky and Colonnier, 1982). Analysis revealed a layer IV in area X that was intermediate in thickness, yet less densely populated than either area 17 or area 18.

Parameters for layers V and VI in area X were indistinguishable from those for Area 17, and significantly different from those for area 18. Furthermore, the separation of layer VI into VIA and VIB sublaminae --seen in 17 but not in 18-- was observed in area X. Layers V and VI provide cortical efferents to subcortical structures, including reciprocal connections to the LGN from Layer IV in the specimen of area 17. Thus, the findings of similar parameters and subdivisions are important.

Thus, laminar analysis added a quantitative dimension to the gross anatomic observations of area X, and allowed for comparison with areas 17 and 18. Area X is characterized by layer-specific properties that distinguish it from both areas 17 and 18. However, it also shares properties in common with both.





## DISCUSSION

These results suggest an interaction between intrinsic area-specific factors of cortical neurons and the influences of extrinsic factors such as thalamic input as postulated in the protomap hypothesis (Rakic, 1988). These results may also suggest that extrinsic factors play a role within the specification and development of cortical areas in a layer-specific fashion.

In this study, the independent variable manipulated was LGN neuron number. There were two critical components, however, to this variable. The first component was the LGN neuron number, which in essence represented both input into area 17, specifically layer IV, as well as target for cortical efferents from layer VI. The second component was the temporal relationship of the manipulation to the stage of cortical development: whether LGN fibers were in the subplate zone or in the cortex at the time of enucleation, and whether cortical neurons destined for specific layers were already generated, were migrating, or were in their final position in the cortex at the time of the manipulation.

The novel cytoarchitectonic area herein described, area X, represents a “hybrid” area in that it displays properties of area 17 in its infragranular layers, including subdivisions of layer VI (see Figure 3-6, Table 3-3). At the same time, the width and neuronal density of supragranular layers likened that of area 18 (see Figure 3-6, Table 3-3). Layer IV, which is the site of major LGN input to area 17, was different from that of areas 17 and 18 in width and neuron density; also, unlike area 17 it was not subdivided into four sublaminae (see Figure 3-6, Table 3-3).

A strong argument may be made that these observations are consistent with the notion of intrinsic specification of boundaries between areas 17 and 18 neuron populations. The surface area of area X corresponded with the complementary reduction of area 17 observed in the E81 and E90 cases as compared with normal controls (see Table 3-2). In support of the previous statement, it appeared in scattered locations as islands within area 17 as well as between the area 17-area 18 border (see Figure 3-5), seemingly redefining a



complement of area 17. Furthermore, this “hybrid” cortex displayed specific patterns of cytoarchitecture in probes examined at different sites (see Table 3-3).

Thus, Area X appears to be a viable cytoarchitectonic area arising from alteration in extrinsic factors, LGN input in this specimen. The timing of the manipulation seemed to be a critical factor in the generation of this area. It appeared only in the subjects undergoing surgical manipulation at midgestation (E81 and E90), which is before invasion of the cortical plate by LGN efferent fibers to cortex. Thus, this area may arise from an alteration in the extrinsic component of area specification. Three plausible mechanisms could be proposed: 1) expansion of thalamo-cortical or cortico-cortical connections that are normally present in small numbers, as in the inferior pulvinar in layers I and II (Benevento *et al.*, 1975) or the superior temporal sulcus (STS) in layers I, IVb, and VI (Rockland and Pandya, 1981); 2) expansion of projections that transiently populate an area but are eliminated by selection or competition, as, again, from the STS, where projections not found in adults were found in newborn macaques (Kennedy *et al.*, 1989); and 3) invasion of projections from adjacent regions such as from area 18. Interestingly, the supragranular layers, which normally receive inferior pulvinar nucleus input and are the site of transient STS projections, were expanded in area X as compared to area 17 (see Table 3-2).

The two earliest binocular enucleations (E59 and E65) were performed when all LGN neurons had been generated and their efferent fibers lie in the subplate zone within the route of migrating cortical neurons; at that time layer IV neurons have not been generated. Notable about these cases was that a great reduction in cytoarchitectonically-normal area 17 was observed, and in one specimen, a small island of area 17 appeared discontinuous from the remainder of area 17 (see Figure 3-2). The E120 specimen displayed normal area 17 surface area and cytoarchitecture, and normal volume, lamination, and total neuron number in LGN. At E120, all thalamo-cortical interactions have been established and all area 17 neurons have been generated. Thus, for all purposes, it served as a control for the technique. It is interesting to note, however, that it had, on gross examination, a reduced



complement of magnocellular layer neurons, when compared to controls. This may be related to the absence of input from the retina, or other factors.

It is possible that similar mechanisms as those involved in respecification of portions of area 17 into area X played a role in the above observations. This is certainly consistent with the finding of an island of area 17 within what appears to be area 18. Indeed, what appears to be area 18 in these cases may be an alteration in the specification of area 17. A clear advantage of this model is the clear distinction of areal boundaries between area 17 and area 18. Respecification of area 17 due to a pattern of projections similar to those in area 18 could make distinctions at the light-microscopic level less clear. Alternate methods used in conjunction with our analysis, such as receptor-binding studies, may add in our ability for distinction and characterization of these areas.

In fact, the cytoarchitecture of the supragranular layers in area X approaches that observed in area 18. Thus, were it not for the distinctions in the infragranular layers it might be difficult to find statistically-significant differences between area X and area 18. It is a possibility that reciprocal connections from layer VI to LGN are already in place or growing towards it prior to elimination of their target. It is not clear whether reciprocal connections from layer VI to thalamus are already established at that time. However, it might be strictly an issue of maturation of the particular cortical lamina; differentiation of layers V and VI begin at approximately E85 (Williams, Ryder, and Rakic, 1987). Thus, the effect of extrinsic factors may be restricted subareas of the cortex as is seen in the barrel fields of rodent somatosensory cortex following removal of vibrissae (Van der Loos and Woolsey, 1973; Killackey *et al.*, 1978) or in mutants with supernumerary vibrissae (Welker and Van der Loos, 1986). These would be superimposed onto a more global effect of intrinsic factors on cortical specification.

Other experiments using this model have also shown reduction of area 17 surface area (Dehay *et al.*, 1989). In these cases, areal boundaries were evaluated grossly on Nissl stain, cytochrome oxidase, and by retrograde labelling of callosal fibers, which do not



normally project to area 17. It was interesting to note that more intense staining than that normally observed in area 18 was observed in enucleates operated earlier than E112. This could represent invasion of intrinsic area 17 devoid of LGN input to generate a novel cytoarchitectonic area.

The concept of a completely extrinsic model (O'Leary, 1989), would predict development of area 18 across areas deprived of normal thalamic input. Thus, area X would not be adequately explained by such a concept. Furthermore, in the early enucleates, one would expect to see discontinuity in area 17 with islands of area 18 invading de-afferented areas.

Recent studies have involved direct thalamic ablation by electrolytic lesion in hamsters (Windrem and Finlay, 1991). They present similar results of alteration of cortical cytoarchitecture, which they attribute to alteration in interactions with afferents. This model has the technical advantage of allowing for direct thalamic ablation, as hamster pups have a relatively less-developed cortex at birth. Unfortunately, there is much trauma to the thalamus associated with the ablation procedure, and input to the cortex from LGN and ventrobasilar nuclei is not as layer-specific as in primates, nor are cortical areal borders as crisp and distinct.

These findings also may have implications in understanding neuropathological conditions. There have been case reports in the literature of bilateral anophthalmos presenting with similar findings in the LGN --decreased volume and lack of lamination-- and in the visual cortex --reduced size and absence of stria of Gennari (Duckworth and Cooper, 1969; Haberland and Perou, 1969; Brunquell *et al.*, 1984).

The protomap model may also be applied to understanding functional studies in the cerebral cortex. Studies using positron-emission tomography in blind humans have demonstrated higher metabolic activity in the visual cortex of those blinded during the perinatal period as compared to later in life (Veraat *et al.*, 1990).





Studies involving experimental alteration of cortical development such as rerouting of cortical afferents for processing in alternate cortical areas (Frost and Metin, 1987; Sur *et al.*, 1988), transplantation of migrating cortical neurons to heterotopic sites in rodents (McConnell, 1985, 1988; O'Leary and Stanfield, 1989), and thalamic ablations in hamster (Windrem and Finlay, 1991) are often difficult to unify due to the alteration of the normal system, and inherent differences in systems. However, they may hold the key to elucidate the roles of extrinsic and intrinsic factors on normal specification of cortical areas, and, on a broader scale, possible mechanisms involved in the evolution of cortical areas.



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